Marine Bioluminescence: Mechanisms and Evaluation

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LONG-TERM GOALS

Our long-term goal is to investigate marine bioluminescence with emphasis on its mechanisms and adaptive significance. The ubiquity of bioluminescence in the sea argues that its importance to marine life far exceeds present understanding. Our efforts center on increasing this understanding through research on a broad front ranging from cellular to populational aspects of bioluminescence.

OBJECTIVES

Objectives during the year have been to: (1) complete work on triggering and adaptive significance of counterillumination in the fish *Porichthys notatus*, (2) complete a multiyear study of bioluminescence in the S. California Bight using moored detectors, (3) continue study of luminescence in gelatinous zooplankton and marine snow, and (4) investigate the role of cytoskeletal elements in the daily migration of luminelles in the dinoflagellate *Pyrocystis fusiformis.*

APPROACH

(1) Counterillumination work involved laboratory experimentation on local *Porichthys* reared in the laboratory from collected nests and on juvenile specimens of the non-luminous population trawled in Puget Sound. Experiments were conducted in our counterillumination apparatus to assess the characteristics of the hydromechanical stimulus essential to induce counterillumination. Anti-predatory effects of counterillumination were tested under appropriate light conditions in large tanks with luminous and non-luminous juvenile fish as prey and adult fish as predators.
(2) Data analysis was the principal effort in the multiyear moored detector study although during part of the year the detectors were still in use.
(3) Data analysis and publication preparation were the principal efforts in the work on gelatinous zooplankton and marine snow.
(4) Cytoskeletal investigations of *Pyrocystis* luminescence were conducted on laboratory cultured material. Some experiments on bulk cultures were done via measurements in the integrating sphere photometer. The most critical work was on micromanipulated single cells visualized with an intensified CCD video microscope system during exposure to agents affecting plant cytoskeletal elements. A time study of the cell cycle with particular regard to luminescence was undertaken.

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WORK COMPLETED

(1) Investigation of the requirement for hydrodynamic stimulation to induce counterillumination in *Porichthys* and the antipredation experiment were completed and submitted for publication as part of the thesis of Robert A. Harper.
(2) Data from the first four years of the long-term bioluminescence study has been analyzed and accepted as part of the UCSB Ph.D thesis of David Lapota.
(3) Analysis of bioluminescence spectra as a function of depth of occurrence was completed and accepted for publication. A manuscript has been completed on luminescence in marine snow as part of the UCSB Ph.D. thesis of Steven Haddock.
(4) Experiments were completed by Carrie McDougall on the role of actin in the reciprocal circadian movements of the light emitting organelles (microsources or scintillons) and the chloroplasts. Preliminary studies basic to investigation of the role of microtubules in this process were completed. Work on bioluminescence and related matters during the cell cycle demonstrated the nature of linking luminescence between newly formed daughter cells

RESULTS

(1) In *Porichthys* investigation of the hydrodynamic stimulus required for counterillumination showed that the most effective broadband stimuli were below 100 Hz, within the hearing range of the fish and approximating the stimulation produced by a fast-turning or accelerating predator. Light emission during counterillumination amounts to about 10% of the light required for complete replacement of light blocked by the fish even though the fish is invisible to the eye, indicating that the photophores, which cover about 8.5% of the ventral surface, are serving to produce an effective disruptive light pattern. An antipredation experiment comparing predation on juvenile luminescent and non-luminescent *Porichthys* showed that counterillumination is clearly protective against attacks by adults of the species. Previously adults of this fish were shown to cannibalize juveniles as, we believe, a mechanism to maintain luciferin levels in the adults made necessary by the fact that only shallow living juveniles have access to the dietary luciferin source, the crustacean *Vargula tsujii*.

(2) Data from the long-term mooring study were analyzed. The data represent four years of once per hour moored instrument measurements from San Diego Bay and 2 years from San Clemente Island pier with periodic transects between the two sites with a shipborne profiling bathyphotometer. Beam attenuation, chrolorophyll, nutrients and temperature data were collected. Of the dinoflagellates found, *Protoperidinium* contributed much of the bioluminescence, but *Ceratium, Gonylaux* and *Noctiluca* were significant under certain conditions. Bioluminescence per cell was measured for netted samples throughout the year and used with specimen number data to construct light budgets for comparison with moored photometer data. Storm water runoff was found to be correlated with increased levels of bioluminescence. Owing to the variation in numbers of heterotrophic and autotrophic dinoflagellates through the year the correlation of bioluminescence with Chl a was not found to be consistent. The work represents the longest such series of data on record.

(3) Bioluminescence of marine aggregates was examined on 4 cruises in the Santa Barbara Channel. Ninety-seven percent of the snow samples were bioluminescent. Aggregates produced several

hundred times the light output of an equivalent volume of surrounding water, with more enrichment occurring on larger particles. Although aggregates represented a miniscule fraction of the water column by volume, they contributed 2 to 44% of stimulated luminescence in the water. Luminescence correlated most strongly with the abundance of dinoflagellates (R^2~0.8)

We examined the variability and potential adaptive significance of the wavelengths of light produced by gelatinous zooplankton. Bioluminescence spectra were measured from 100 species of planktonic cnidarians and ctenophores collected between 1 and 3500 m depth. Species averages of maximal wavelengths for all groups ranged from 440 to 506 nm. Ctenophores (41 species) had characteristically longer wavelengths than medusae (34 species), and the wavelengths from siphonophores (25 species) had a bimodal distribution across species. Four species each produced two different wavelengths of light, and in the siphonophore *Abylopsis tetragona* these differences were associated with specific body regions.

(4) In *Pyrocystis* the actin cytoskeleton was found to radiate to the periphery of the cell from the central nucleus, following the path of the migrating luminescent organelles. Cytochalasin-D, which caps the growing ends of actin filaments, disrupts circadian movement of both chloroplasts and luminescent organelles. It has been postulated that the movement of the scintillons out of the periphery represents a decoupling with the bioluminescent stimulation mechanism and thus, these circadian movements may be the key to the control mechanism. Experiments were completed involving the examination of actin's role in the bi-directional circadian movement of the light-emitting organelles, (microsources or scintillons), and the chloroplasts. Cells were treated with CD in one phase of their light-dark cycle and examined 4-5 hrs. later in the subsequent phase. 50% of the treated cells had both chloroplasts and scintillons distributed in patterns that reflected the previous phase of the light cycle, i.e. the organelles had not been able to migrate to the proper position associated with the current light cycle phase.

Further observations of the scintillon movements in normal cells compared to cells with disrupted actin showed that the scintillons do appear to be synthesized around the nuclear region at the end of day phase and transported to the periphery just before night fall. Also observed was that cells treated with CD in night phase and examined in day phase had scintillons that remained in the periphery throughout the entire day phase, indicating that the scintillons are not broken down in the periphery and that they do move back to the nuclear region at the end of night phase. Still under investigation is whether these day phase peripheral scintillons are mechanically stimulable. Conclusions drawn from these studies are that the nuclear area appears to be the hub for recycling, synthesis, and packaging of the components of the scintillons and that the new scintillons are moved from this area to the periphery each night and then moved back to this area at the end of each night.

Examination of bioluminescence in dividing cells has provided new insights into the reproduction of *P. fusiformis*. When mechanically stimulating individual cells with two clones in one cell wall it was discovered that the flash propagates from one clone to the other. This propagation takes approximately 67 msec. Additionally, it was found that one clone appeared to contain a flash initiation center, in that every time the cell was stimulated the same clone flashed first and then the flash propagated to the other clone. This occurred regardless of where on the cell the stimulation

occurred and with repeated stimulation. This indicates that the clones maintain an electrophysiological connection with each other right up until they break free of the old cell wall.

IMPACT/APPLICATIONS

The counterillumination study is the first example of the dependence of such behavior on a stimulus modality in addition to the level of background light. We believe this might be a consequence of the fact that this fish must obtain its luciferin in the diet, thus rendering the supply limited. This work is also the first experimental demonstration of the antipredation effect of counterillumination. Juvenile *Porichthys* are shown to be excellent subjects for studies on counterillumination.

The long-term investigation of dinoflagellate coastal bioluminescence provides valuable information on the detailed interrelations of luminescent populations and the effects of environmental parameters on their luminescent potential. The work is of interest in respect to red tides, which often include luminescent species, and might have bearing on naval coastal operations.

Although the marine snow we examined was mainly formed by the aggregation of diatoms, which do not produce light, the accumulation of dinoflagellates and other luminous organisms caused the particles to be brightly luminescent. And while our results support previous findings that in coastal waters light is mainly produced by dinoflagellates, the clumped small-scale distribution we found differs from the general assumption that luminescent sources are distributed homogeneously. Marine aggregates are sites of enhanced bioluminescence, and this may strongly affect how organisms interact with them, in turn altering flux rates.

In the gelatinous zooplankton, light from deep-dwelling species had significantly shorter wavelengths than light from shallow species in both ctenophores ($p = 0.010$) and medusae ($p = 0.009$), indicating a quantitative correlation between habitat and the evolution of color. The species which emit blue and green light may hold clues to how luminescence colors are selected for, but this awaits further investigation. Although light production in these organisms was limited to the blue-green wavelengths, it appears that within this range, colors are well-adapted to the particular environment which the species inhabit.

Cellular mechanisms controlling triggering of bioluminescence in any organism are hardly known. Our work shows that the large dinoflagellate *Pyrocystis fusiformis* is a useful system for research in this area. So far the work has shown dependence on cytoskeletal structures to regulate the circadian aspect of luminescence. It is anticipated that further research will reveal the linkage between mechanical excitation and light emission in this organism using techniques worked out thus far in the investigation.

TRANSITIONS

Our work on coastal luminescence is contributing to development of protocols for monitoring bioluminescence in relation to naval operations by D. Lapota , Space and Naval Warfare Systems Center, San Diego, CA.

The remainder of the work under this grant is devoted to solving questions about mechanisms and adaptive significance of marine bioluminescence that contribute to understanding the significance of this widespread phenomenon to population biology in the sea.

PUBLICATIONS

Haddock, S.H.D. and Case, J.F. (in press): Bioluminescent spectra of shallow and deep-sea gelatinous zooplankton: medusae, ctenophores and siphonophores. Marine Biology.

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